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Simultaneous estimation of water soluble vitamin by high performance liquid chromatography (HPLC) method in five wild edible plants consumed by the tribal people of North-Eastern region in India

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Abstract
Phyllostachys mannii, *Litsea cubeba*, *Polygonum chinense*, *Musa cheesmanii* and *Musa flaviflora* are potent wild edible plants and consumed by the tribal people of North-eastern region in India. A reversed-phase high-performance liquid chromatographic method using photodiode array detector with gradient elution has been developed and validated for the simultaneous quantitation of several water-soluble vitamins like ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6) and folic acid (vitamin B9) in these five wild edible plants. The chromatographic separation of vitamins were carried out on Acclaim C 18 column (5 µm particle size, 250 x 4.6 mm), Dionex Ultimate 3000 liquid chromatograph and detection was carried out at three different wave lengths (210, 245 and 254 nm) using a mobile phase of acetonitrile and aqueous trifluoro acetic acid (TFA, 0.01% v/v) solution with gradient elution. The results of investigation showed that these plants are rich sources of vitamins, especially the B-group of vitamins that can contribute immensely to nutrition, food security and health and therapeutic benefits. The high percentage of recovery (98-99%), low coefficient of variation ($R^2 > 0.99$) and low limit of detection (LOD) and limit of quantitation (LOQ) confirm the suitability of the method for simultaneous quantification of vitamins in these five plants under investigation.

Keywords: Wild edible plants, Water soluble vitamins, B group vitamins, Vitamin C, HPLC Analysis

1. Introduction
Vitamins are basic elements of nourishment which are required in modest quantities in the body all the time to lead typical wellbeing and different physiological capacities in the human body. They are broadly circulated in normal nourishment sources and can be effectively acquainted into the weight control plans with fulfil every day needs. Vitamins are a gathering of organic compounds and can be sorted into two gatherings dependent on their solvency: fat-solvent vitamin and water-dissolvable vitamin. The previous incorporates lipid dissolvable vitamins A, D, E, and K and different carotenoids, the last is made out of water solvent vitamins C and eight B-vitamins, in particular thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic corrosive (B5), biotin (B7), folate (B9) and cyanocobalamin (B12) [1].

Estimation of vitamins in foods is confounded by numerous components. It is exceptionally hard to build up a solitary widespread strategy for the concurrent evaluation of nutrient because of their assorted substance structures and properties. In addition, every vitamin can happen in various structures considered vitamers that have the equivalent biological activity upon ingestion. Vitamins frequently happen in food at moderately low levels and powerless to debasement by presentation to light, air, warmth and high pH. Distinctive instrumental techniques have been utilized for the assurance of Vitamin C and B-bunch nutrients, including spectrophotometry, titration, High performance liquid chromatography (HPLC), capillary electrophoresis (CE), High performance thin layer chromatography (HPTLC) and microbiological examines have been accounted for the assurance of water-dissolvable nutrients in different conditions. The most broadly utilized techniques for the assurance of ascorbic acid together with B-bunch nutrients are turned around stage HPLC combined with diode array detector, utilizing a C18 column and aqueous–organic mobile phases, in acidic media [2].

Plants wealthy in natural products, vegetables, entire grains, and vegetables, give a bounty of nutrients and minerals to meet one's nourishing needs. The helpful capabilities of the vegetables are to a great extent reliant on the nearness of indispensable nutrients just as micronutrients. Despite the fact that vitamin is required a modest quantity for every day in wellbeing, it assumes a fundamental job in our wellbeing.

The utilization of verdant vegetables and fruits plentiful in nutrients, are accounted for to lessen the danger of assault of different intense and incessant infections [3].

The wild plants have been a fundamental wellspring of nourishment and medication for inborn individuals. These plants have rich sustenance and restorative qualities. Ordinary utilization of vegetables is additionally prescribed for better wellbeing and the board of interminable sicknesses. The nutritive worth, antioxidant properties of the products of wild palatable plants like *Phyllostachys mannii*, *Litsea cubeba*, *Polygonum chinense*, *Musa cheesmanii* and *Musa flaviflora* devoured by the inborn individuals of North-eastern area in India has just been concentrated in our research facility.

Along these lines, these wild consumable plants has nourishing potential and are deserving of abuse as a dietary asset because of the nearness of adequate measure of protein, starch, fat and minerals. The cell reinforcement properties and the nearness of phenolic acids and flavonoids in these wild palatable plants in fluctuating sums have been advanced the nutraceutical properties of these plants [4].

This paper accounts a simple, gradient and stability-indicating HPLC method for the rapid determination of water soluble vitamins like, thiamine (B1), niacin (B3), pyridoxine (B6), ascorbic acid (C), pantothenic acid (B5), riboflavin (B2) and folic acid (B9) in five wild edible plants named *P. mannii*, *L. cubeba*, *P. chinense*, *M. cheesmanii* and *M. flaviflora* from North-eastern region in India and all the vitamins were simultaneously analyzed in a single chromatographic run.

2. Materials and Methods

2.1 Plant material

The wild edible plants named *Phyllostachys mannii* Gamble. (Family: Poaceae), *Litsea cubeba* (Lour.) Pers. (Family: Lauraceae), *Polygonum chinense* L. (Family: Polygonaceae), *Musa cheesmanii* N. W. Simonds (Family: Musaceae) and *Musa flaviflora* N. W. Simonds (Family: Musaceae) were collected from North-eastern region in India. It was duly authenticated and a voucher specimen was kept at the Department of Plant Chemistry of Botanical Survey of India under the Registry No. BSITS 81, BSITS 82, BSITS 83, BSITS 84 and BSITS 85 for future reference. The plant parts were taken in our laboratory at refrigerated temperature using cold packs. The refrigerated plant samples were stored at -15 ^{0}C and then processed within four days of collection.

2.2 Chemicals

The standards chemicals like ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the HPLC-grade solvents such as acetonitrile, methanol, water sodium dihydrogen phosphate and trifluoroacetic acid were purchased from Merck (Germany).

2.3 HPLC equipment

HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 μl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim ^{TM} 120 C18 column (5 μm particle size, i.d. 4.6 x 250 mm).

2.4 Preparation of standard solutions

The stock standard solutions of vitamin C, B1, B3, B5 and B6 and were prepared by dissolving 25 mg of the each standard in one ml 0.1 M hydrochloric acid in 25 ml standard volumetric flask and topped up to mark with double distilled water. For preparation of standard stock solutions of vitamin B9 and B2, 25 mg of the each standard were dissolved in one ml 0.1 M sodium hydroxide in 25 ml standard volumetric flask and made up to mark with double distilled water. The standard solution was stored in amber-glass bottles in the refrigerator at 4 ^{0}C. The working standards were prepared from the stock standard solutions by mixing 100 µl mixed vitamins standard (vitamin B9, B5 and B2), 800 µl phosphate buffer (1 M, pH 5.5) and 100 µl mixed vitamins standard (vitamin C, B1, B6 and B3) which represent 100 µg/ml mixed working standards. The working standard solutions of concentrations 20, 40, 60 and 80 µg/ml were prepared accordingly.

2.5 Preparation of sample solution

Plant materials were cleaned and the inedible portions were removed. The edible parts were rinsed thoroughly with tap water and then with distilled water. The washed plant materials were dried with clean cloth, were cut into very small pieces, frozen in liquid nitrogen, freeze-dried and kept at -20 ^{0} C until analysis.

One gm of each freeze-dried plant materials were soaked in 10 ml water. Then 1 ml 0.1 M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in dark for 24 hours. The solution was first filtered through a Whatman No. 1 filter paper and the resulting filtrate was taken in a 25 ml volumetric flask and solution was topped up to the mark with HPLC grade water. The sample solution was filtered through 0.45 μm membrane filter before injection into LC system. The stock solutions of sample were kept in a refrigerator for further use.

2.6 Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by Seal et al 2018 [5] with minor modification. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (Solvent B), the column was thermostatically controlled at 22^0 C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elusion was 1 % A and 99 % B with flow rate 0.5 ml/min in 5 min, from 1 % to 25% A with flow rate 0.5 ml/min for 16 min, 45 % A, with flow rate 0.5 ml/min for 8 min. from 45 to 1 % A with flow rate 0.5 ml/min in 5 min. The mobile phase composition back to initial condition (solvent A: solvent B: 1: 99) in 34 min and allowed to run for another 1 min, before the injection of another sample. Total analysis time per sample was 35 min.

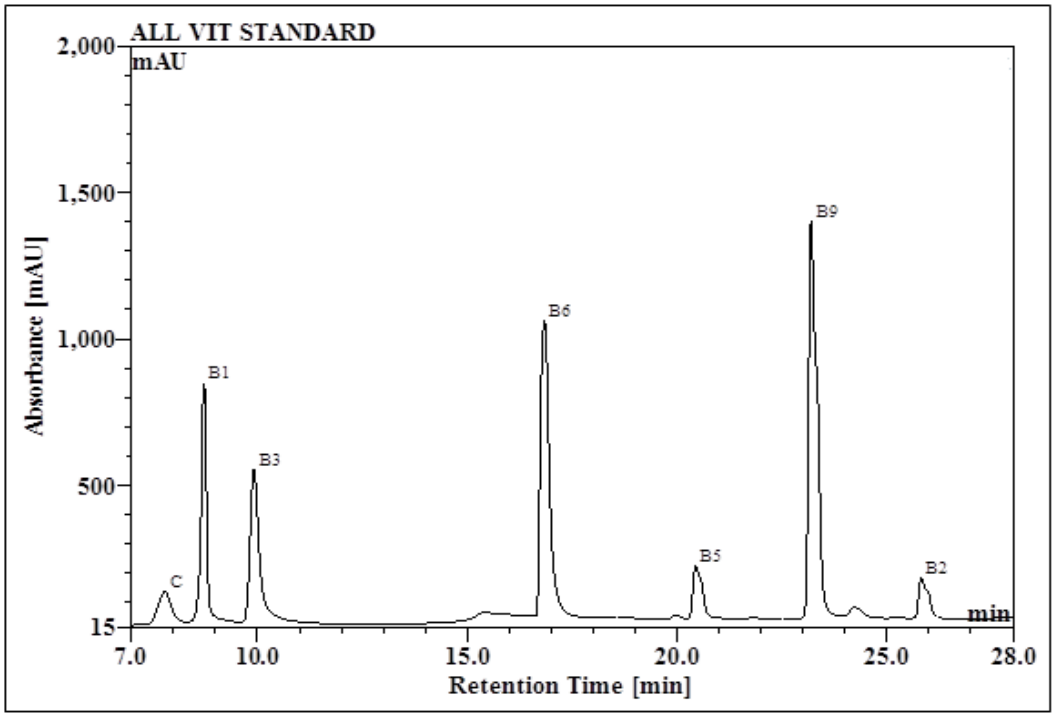
The various concentrations of (20, 40, 60, 80 and 100 µg/ml) vitamin working standards were injected into the HPLC column separately and the retention times were noted and used to identify the vitamins in the sample.

HPLC Chromatograms of all vitamins were detected using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm) according to absorption maxima of analysed compounds. Each compound in the plant extracts were identified by its retention time and by spiking with standards under the same conditions.

The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported as means ± standard error means of three independent analyses and the method was validated according to the USP and ICH guidelines [6-7]. Various parameters were studied to validate the reproducibility of the method *viz.* the effectiveness, the linearity, the limit of detection (LOD), the limit of quantitation (LOQ), the precision and the accuracy.

2.7 Statistical analysis
The significant and non-significant variations within water soluble vitamin contents and the five wild edible plants were analyzed using one-way analysis of variance (ANOVAs). All the investigation was completed utilizing triplicate tests. Test results were exposed to univariate analysis of variance (ANOVA), trailed by Tukey test ($p \leq 0.05$) utilizing the statistical package for the social sciences (SPSS variant 7.5).

3. Results
3.1 Chromatographic method
A typical HPLC chromatogram of the all standard vitamin mixture recorded at 210 nm is presented in Figure. 1. As shown in the chromatogram, all investigated compounds had responses at 245 nm, where they were successfully separated. The constituents under investigation were also identified by the recorded absorption spectra, which were comparable both for plant extracts and standard substances. The regression coefficient together with LOD and LOQ values, are shown in Table 1. The high value of $R^2 > 0.9906$ in the range of analyzed concentrations at 210, 245 and 275 nm is indicative of responsive linearity.



C: Ascorbic acid; B_1: Thiamine; B_3: Niacin; B_6: Pyridoxine; B_5: Pantothenic acid; B_9: Folic acid; B_2: Riboflavin

Fig 1: HPLC Chromatogram of mixture of Standard vitamin

Table 1: Retention time and parameters of calibration curve, precision and repeatability, LOD, LOQ and percent recovery study of standard water soluble vitamins for HPLC method validation

Name of the Standard Vitamin	Detected at wavelength λ nm	Retention time	RSD (%) of the retention time	RSD (%) of the peak area at conc 40 µg/ml	RSD (%) of the peak area at conc 60 µg/ml	Regression Coefficient R^2	LOD µg/ml	LOQ µg/ml	Percentage of recovery (%)
Vitamin C	245	7.79	0.956	0.138	0.149	99.88	0.186	0.565	98.76
Vitamin B1	245	8.73	0.462	0.025	0.032	99.73	0.034	0.103	98.24
Vitamin B3	245	9.92	0.706	0.206	0.171	99.83	0.277	0.839	98.50
Vitamin B6	275	16.84	0.712	0.799	0.382	99.91	1.062	3.219	98.15
Vitamin B5	210	20.44	0.830	0.173	0.103	99.89	0.233	0.705	98.33
Vitamin B9	275	23.19	0.475	0.220	0.227	99.10	0.309	0.935	99.20
Vitamin B2	275	25.82	0.453	0.114	0.144	99.68	0.156	0.472	98.25

Note: RSD Relative standard deviation, LOD Limit of detection, LOQ limit of quantification

3.2 Identification and quantification of water soluble vitamins in the wild edible plants:
The HPLC method was successfully performed for the estimation of water soluble vitamin e.g ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9). The quantity of all vitamins of all plant materials has been expressed as mg/100gm dry plant material and data presented in table 2.
The HPLC analysis of the plants *P. mannii* showed the presence of vitamin B1 (0.14±0.004 mg/100 gm), B2 (0.25±0.003 mg/100gm), B3 (0.04±0.005 mg/100gm), B5 (0.58±0.01 mg/100 gm), B6 (0.32±0.004 mg/100 gm) and B9 (0.08±0.004 mg/100gm).

Table 2: Quantification of Vitamin C and B1, B2, B3, B5, B6 and B9 in five wild edible plants

Vitamin content in mg/ 100 gm dry plant material							
Plant	**Vitamin C**	**Vitamin B1**	**Vitamin B2**	**Vitamin B3**	**Vitamin B5**	**Vitamin B6**	**Vitamin B9**
P. mannii	ND	0.14±0.004^{c}	0.25±0.003^{c}	0.04±0.005^{d}	0.58±0.01^{a}	0.32±0.004^{c}	0.08±0.004^{c}
L. cubeba	86.85±0.54^{b}	0.22±0.02^{b}	1.49±0.02^{b}	0.28±0.02^{a}	0.33±0.03^{b}	1.34±0.05^{a}	0.09±0.004^{b}
P. chinense	90.09±0.07^{a}	0.26±0.005^{a}	4.57±0.02^{a}	0.25±0.004^{b}	0.11±0.006^{e}	0.75±0.004^{b}	0.04±0.003^{e}
M. cheesmanii	ND	0.04±0.002^{d}	0.12±0.008^{d}	0.05±0.001^{c}	0.22±0.004^{c}	0.28±0.004^{c}	0.05±0.003^{d}
M. flaviflora	ND	0.04±0.0006^{d}	0.24±0.004^{c}	0.05±0.0004^{c}	0.18±0.004^{d}	0.15±0.004^{d}	1.95±0.008^{a}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at 95% confidence level and statistical significance were accepted at the $p<$ 0.05 level. The superscript letter a, b, c, d and e denotes the significant differences within same parameters of individual plant

The HPLC study of the plant *L. cubeba* revealed the presence of vitamin C (86.85±0.54 mg/100gm) B1 (0.22±0.002 mg/100gm), B2 (1.49±0.02 mg/100gm), B3 (0.28±0.02 mg/100gm), B5 (0.33±0.03 mg/100 gm), B6 (1.34±0.05 mg/100 gm) and B9 (0.09±0.004 mg/100gm).

The presence of vitamin B1 (0.26± 0.005 mg/100 gm), B2 (4.57±0.02 mg/100gm), B3 (0.25±0.004 mg/100 gm), B5 (0.11±0.006 mg/100 gm), B6 (0.75±0.004 mg/100gm), B9 (0.04±0.003 mg/100gm) and remarkable amount of Vitamin C (90.09±0.07 mg/100gm) were detected in *P. chinense*.

The inflorescence of *M. cheesmanii* were found to contain vitamin B1 (0.04±0.002 mg/100gm), B2 (0.12±0.008 mg/100gm), B3 (0.05±0.001 mg/100 gm), B5 (0.22±0.004 mg/100 gm), B9 (0.05±0.003 mg/100gm) and significant amount of B6 (0.28±0.004 mg/100gm).

Our investigation disclosed the occurrence of vitamin B1 (0.04±0.0006 mg/100gm), B2 (0.24±0.004 mg/100gm), B3 (0.05±0.0004 mg/100gm), B5 (0.18±0.004 mg/100 gm), B6 (0.15±0.004 mg/100 gm) and good amount of B9 (1.95±0.008 mg/100gm) in the inflorescence of *M. flaviflora*.

4. Discussion

4.1 Chromatographic method

The quantitative analysis of water soluble vitamins were carried out using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm). The detection of vitmain C, B1 and B3 were carried out at wavelength 245 nm, vitamin B2, B6 and B9 were carried out at 275nm. The detection wavelength was set at 210 nm for vitamin B5 as it showed absorption at 209 nm. The chromatographic separation was performed at a flow rate of 0.5 ml/min. The method proposed was rapid and all analytes were completely eluted within 30 min and the whole chromatographic run was completed in 35 min. The solvent system (acetonitrile and aqueous trifluoro acetic acid (TFA, 0.01% v/v) was used for the analysis and produced a sharp peak of the studied vitamins.

The repeatability of the retention time for all the standard samples and the relative standard deviation for the peak areas of two standards *viz*., 40 µg/ml and 60 µg/ml was found to be below one percent. The significantly high rate of recovery (98.15 – 99.20%) of the standard vitamins worth's mention. It follows that the method under consideration is characterized by precision, accuracy, meticulousness and can be used for the qualitative as also quantitative estimation of water soluble vitamins in the five wild edible plants under investigation.

The aim of this study was to develop simple, gradient, and stability-indicating HPLC method for the determination of Vitamin C, B1, B2, B3, B5, B6 and B9 in five wild edible plants. Vitamin C is extremely unstable in basic and neutral solutions, but relatively stable in acidic solutions, therefore phosphate buffer (pH 5.5) was used as a diluting solution for vitamin C, B1, B3, B5 and B6. Both the vitamins (B2 and B9) were found slightly soluble in water and acidic aqueous solutions, but soluble in basic aqueous solutions. So the stock solutions of vitamin B2 and B9 were dissolved in 0.1M NaOH solution and all working standard vitamins were diluted with phosphate buffer (pH 5.5) solution.

4.2 Identification and quantification of water soluble vitamins in the wild edible plants

Vitamin C is the most significant nutrient in foods grown from the ground. It is notable for its cell reinforcement properties and it helps the body in repressing from viral disease, bacterial contaminations and poisonous quality. It is required for the avoidance of scurvy and upkeep of solid skin, gums and veins and the inadequacy of this nutrient causes wounding, bleeding, dry skin and sadness [8].

The experimental result showed that, the amount of vitamin C was found highest in the leaves of *P. chinense* (90.09 ± 0.07 mg/100gm) followed by in the fruits of *L. cubeba* (86.85±0.54 mg/100gm) (Table 2). The vitamin C content in these wild edible plants are very much comparable with some common fruits and vegetables like *Solanum tuberosum* (17.04± 1.18 mg/100gm), *Allium sativum* (13.06 ± 1.10 mg/100gm), *Daucuscarota sativus* (2.55± 0.72 mg/100gm) etc. [8]. Vitamin C was not detected in other plants under investigation.

So the wild consumable plants under scrutiny may be viewed as great wellsprings of vitamin C, also, in this way, might fulfill the suggested day by day remittances (RDA) of 75 mg/day and 90 mg/day for grown-up ladies and men separately, and 45 mg/day for offspring of 9–12 years of age. Due to having cell reinforcement properties, vitamin C rich plant may be helpful to decrease the danger of atherosclerosis and a few types of malignant growth [9].

Thiamine (B1), is a basic supplement required by the body for keeping up cell work and thus a wide exhibit of organ capacities. It is irreplaceable for vitality generation, starch digestion and nerve cell work. The lack of this nutrient prompts discount degeneration of the body, especially the anxious and circulatory frameworks, hypertension and heart ailments [10-11].

The thiamine content in these wild edible plants ranged from 0.04±0.0006 to 0.26±0.005 mg/100gm. The highest amount of B1 was obtained from the leaves of *P. chinense* followed by *L. cubeba* and *P. mannii.* (Table 2).

Thiamine has been shown to occur in some common vegetables and fruits like apple (0.016 mg/100gm), beans (0.132mg/100gm), cauliflower (0.073 mg/100gm), spinach (0.076mg/100gm) etc and these amounts are very much similar to the thiamine content detected in the wild edible fruits under investigation.

Riboflavin (B2) is a fundamental nutrient required for appropriate vitality digestion and a wide assortment of cell forms. It is the partner to thiamine utilized in the fortifying of nourishment items [12]. A critical variety of riboflavin content

was seen among the tried wild consumable natural products. The highest amount of B2 was detected in the leaves of *P. chinense* (4.57±0.02 mg/100gm) and the least amount was detected in *M. cheesmanii* (0.12±0.008 mg/100gm). The fruits of *L. cubeba*, inflorescence of *M. flaviflora* and young shoots of *P. mannii* were also found to contain a very good quantity of vitamin B2 (Table 2) which are comparable with some common fruits and vegetables like almonds (1.10 mg/100g), spinach (0.24 mg/100g), beet greens (0.41 mg/100g), green beans (0.12±2 mg/100g, potato (0.023 ±1 mg/100g) etc. [13]

The niacin (B3) content in the wild edible fruits under analysis ranged between 0.05±0.0004 mg/100gm to 0.28±0.02 mg/100gm. The highest amount of B3 was detected in *L. cubeba* followed by in the leaves of *P. chinense* (0.25±0.004 mg/100gm) (Table 2). Therefore the edible parts of these plants are the important sources of vitamin B3, which were comparable with cabbage, cauliflowers, cucumber, spinach, tomatoes ranged between 0.19 -0.97 mg/100gm [13].

Vitamin B3, is a significant nutrient required for handling fat in the body, bringing down cholesterol levels, and managing glucose levels. It is significant in DNA fix, Ca digestion, intracellular breath, and biosynthesis of unsaturated fat and steroids [14]. So the normal utilization of these natural products would supply satisfactory B3 important to keep up solid body capacities.

Vitamin B5, or Pantothenic acid, is a basic nutrient required by the body for cell forms and ideal upkeep of fat. The inadequacy of nutrient B5 prompts fractiousness, weariness, aloofness, deadness, paresthesia, and muscle issues in individual [15].

Pantothenic acid was detected highest in the young shoots of *P. mannii* (0.58±0.01 mg/100gm). The edible parts of *L. cubeba*, *P. chinense*, *M. cheesmanii* and *M. flaviflora* were also found to contain a very good amount of B5 (Table 2).

Pyridoxine (B6) is another water solvent vitamin fundamental for the best possible support of red platelet digestion, the sensory system, the safe framework, and numerous other real capacities. It likewise assumes a job in homocysteine manufactured and degradative responses [16]. This nutrient is found in most nourishment items and furthermore, because of its stabilty, is regularly utilized for strengthening nourishment items [17]. It was measured in all the wild eatable plants under our examination. The highest B6 was observed in the fruits of *L. cubeba* (1.34±0.05 mg/100gm) whereas the minimum was detected in *M. flaviflora* (0.15±0.004 mg/100gm). An appreciable amount of B6 was detected in other plants under investigation (Table 2). The amount of B6 obtained in these wild edible fruits were comparable with some common vegetable and fruits like banana (0.37 mg/100g), avocados (0.29 mg/100g), spinach (0.24 mg/100g), broccoli (0.134 mg/100g), cauliflower (0.115mg/100g), cucumber (0.2 mg/100g) etc. So the regular intake of these plants would supply sufficient B6 necessary to maintain healthy body functions.

Vitamin B9 (folic acid) is a water-solvent B vitamin with numerous rich characteristic sources. It is required for various body capacities including DNA combination and fix, cell division, and cell development. The lack of folate can prompt pallor in grown-ups, and more slow improvement in kids [18-21]. It assumes a significant job as cell reinforcement *in vivo*, both by averting the unfriendly impact of receptive oxygen species (ROS), just as by repressing lipid peroxidation [22]. The extent of B9 in five wild edible plants ranged from 0.04±0.003 to 1.95±0.008 mg/100gm. The content of B9 was found highest in *M. flaviflora* and a good amount of B9 was also detected in other plants under investigation (Table 2).

5. Conclusion

The reversed-phase HPLC strategy with diode array detection was created for the quantitative estimation of water solvent B vitamins (B1, B2, B3, B5, B6 and B9) and vitamin C in five wild palatable plants like *P. mannii*, *L. cubeba*, *P. chinense*, *M. cheesmanii* and *M. flaviflora* gathered from North-eastern area in India. The established HPLC test indicated a well separation of the compounds and furthermore the created technique was linear, sensitive, accurate, meticulous and reproducible. In this manner, the strategy can be utilized for the concurrent assurance of water dissolvable B vitamin and vitamin C in various plans with 'shorter run time' and 'high effectiveness'. RP-HPLC results indicated the plants contained a few water dissolvable B and C nutrients in varying amounts. The aftereffect of examination of nutrient substance in the wild palatable plants under scrutiny will fill in as a valuable way to compute dietary admission of C and B nutrients in the all inclusive community. These information will likewise be useful in the readiness of a total nourishment creation table for healthful overview and furthermore for other research purposes.

6. Conflict of interest

We have no conflict of interest.

7. Acknowledgement

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